Jurnal Teknologi

Full paper

Potential of Tissue Cultured Medicinal Plants in Malaysia

Zahidah Ayob[a], Alina Wagiran[a], Azman Abd Samad[a*]

[a]*Department of Biotechnology and Medical Engineering, Faculty of Biosciences and Medical Engineering, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia*

*Corresponding author: azmansamad@utm.my

Article history

Received :1 August 2012
Received in revised form :
1 January 2013
Accepted :15 April 2013

Graphical abstract

Abstract

Medicinal plants possess many secondary products that exhibit biological activities such as antioxidant, anticancer, anti-inflammatory, antibacterial and anti microbial. Scientific findings have demonstrated that tissue culture techniques could be an alternative tool to propagate plant *in vitro* and manipulate secondary metabolites in medicinal plants. This review aims to give an update on the various plant regeneration of some locally used medicinal plants in Malaysia such as *Eurycome longifolia* Jack, *Zingiber officinale* Roscoe, *Centella asiatica* L., *Justicia gendarussa* Burm. f, *Kaempferia galanga* L. and *Orthosiphon stamineus* Benth. Different type of cultures including organ, callus and cell cultures is also discussed.

Keywords: Medicinal plants; plant growth regulator; *eurycoma longifolia*; *zingiber officinale*; *centella asiatica*; *justicia gendarussa*; *kaempferia galangal*; *orthosiphon stamineus*

Abstrak

Tumbuhan ubat-ubatan mempunyai banyak produk-produk sekunder yang mempamerkan aktiviti-aktiviti biologi seperti anti-pengoksidaan, anti-kanser, anti inflamasi, anti-bakteria dan anti-mikrob. Penemuan saintifik menunjukkan bahawa teknik kultur tisu boleh digunakan sebagai kaedah alternatif untuk pembiakan tumbuhan secara *in vitro* dan manipulasi metabolit sekunder pada tumbuhan ubat-ubatan. Tinjauan ini bertujuan memberikan perkembangan terkini berbagai teknik regenerasi sebahagian daripada tumbuhan ubat-ubatan di Malaysia seperti *Eurycome longifolia* Jack, *Zingiber officinale* Roscoe, *Centella asiatica* L., *Justicia gendarussa* Burm f, *Kaempferia galanga* L. dan *Orthosiphon stamineus* Benth. Jenis-jenis kultur seperti kultur organ, kalus dan sel juga turut dibincangkan.

Kata kunci: Tumbuhan ubat-ubatan; pengawalturan tumbesaran tumbuhan; *eurycoma longifolia*; *zingiber officinale*; *centella asiatica*; *justicia gendarussa*; *kaempferia galangal*; *orthosiphon stamineus*

1.0 INTRODUCTION

Many drugs in the modern day are derived from plants (Manoj *et al*., 2011). Medicinal plants are highly demand due to their valued in food industries, cosmetic industries, pharmaceutical industries and a subset of the national biodiversity wealth (Suneetha and Chandrakandh, 2006). Medicinal plants contain many compounds that have important roles such as antibacterial (Karthikeyan *et al*., 2009; Jothimanivannan *et al*., 2010), antimicrobial (Bhat and Karim, 2010), antioxidant (Purnomo *et al*., 2010; Jothimanivannan *et al*., 2010), antitumor (Susanti *et al*., 2008), antimalarial (Bhat and Karim, 2010), anti-inflammatory (Tiwari *et al*., 2000; Karthikeyan *et al*., 2009; Forkman, 1991) and anti-analgesic (Jothimanivannan *et al*., 2010). Plant secondary compounds are classified according to their biosynthetic pathways. Three major molecule families are phenolics, terpenes and alkaloids. For example, a widespread metabolite, phenolics are involved in lignin synthesis (Bourgaud *et al*., 2001). Table 1 summarizes bioactive compounds identified in local medicinal plants.

Plant tissue culture has become a valuable tool to elucidate secondary metabolites biosynthesis pathway and production of plant products in pharmaceutical industries. For example, *in vitro* techniques of medicinal plants such as callus culture could be used to maximize bioactive compounds production (Rafidah *et al*., 2004). There are many approaches to propagate medicinal plants *in vitro*. Micropropagation of medicinal plants has been documented such as using shoot tips/ axillary buds via organogenesis (Rout *et al*., 2000), production of adventitious shoot (Thomas and Yoichiro, 2010) and somatic embryogenesis (Omar *et al*., 2004). Examples of regeneration studies of local medicinal plants are shown in Table 2.

There are many factors that affect plant regenerations which include carbon source (Reza *et al*., 2009), types and concentration of various plant growth regulators (Kavyashree, 2009), explants and types of media used (Omar *et al*., 2004). Therefore, this paper intended to summarize plant regeneration system of Malaysian medicinal plants such as *Eurycome longifolia* Jack, *Zingiber officinale* Roscoe, *Centella asiatica* L.,

Justicia gendarussa Burm. f, *Kaempferia galanga* L. and *Orthosiphon stamineus* Benth.

Table 1 Bioactive compounds of some medicinal plants locally found in Malaysia

Types of plants	Bioactive compounds	Medicinal properties	References
Eurycoma longifolia (Tongkat Ali)	eurycomaoside, eurycolactone, eurycomalactone, eurycomanone, alkaloid, quassinoids	Antimalarial, aphrodisiac, anti-diabetic, antimicrobial, anti-pyretic	Bhat and Karim, 2010
Zingiber officinale (Halia)	Zingeron, (6)-gingerol, (6)-shogaolmethyl ester, 9-octadecenoic, nortrachelogenin	Antioxidant	Purnomo *et al.*, 2010
Centella asiatica (Pegaga)	indocentelloside, brahmoside, brahminoside, asiaticoside, theankuniside isothankuniside	Antibacterial, anti-inflammatory, anti- febrile, galactogogic	Tiwari *et al*., 2000 ; Karthikeyan *et al.*, 2009
Justicia gendarussa (Gandarusa)	Flavonoid, quercetin, myricetin anthocyanins, flavonols, flavones, chalcones, aurones	Antioxidant, anti-inflammatory, anti-analgestic	Jothimanivannan *et al.*, 2010 ; Forkman (1991)
Kaempferia galanga (Cekur)	α-pinene, camphene, carvone, benzene, eucalyptol,borneol, methyl cinnamate, pentadecane, ethyl-*p*-methoxycinnamate	anticancer, anti-monoamine oxidase	Tewtrakul *et al.*, (2005)
Orthosiphon stamineus Benth. (Misai kucing)	Phenol, flavonoids	anti-diuretic, anti-bacterial	Schut and Zwaving, (1993)

Table 2 Plant regeneration studies of some medicinal plants

Type of plants	Explants source	Organs	Medium + PGR	References
Eurycoma longifolia (Tongkat Ali)	Leaf	Callus	MS + 1.0 mg/l 2,4 -D	Mahmood *et al*., 2010
	Petiole, Cotyledon	Callus	MS + 4.0 mg/l 2,4-D	Mahmood *et al*., 2010
	Rachis	Callus	MS + 4.0 mg/l picloram	Mahmood *et al*., 2010
	Stem, Embryo	Callus	MS + 2.0 mg/l 2,4-D	Mahmood *et al*., 2010
	Tap root	Callus	MS + 3.0 mg/l 2,4-D, MS + 1.0 mg/l picloram	Mahmood *et al*., 2010
	Leaf	Shoot	MS + 5.0 mg/l Kn	Hussein *et al*., 2005
	Shoot tips	Root	MS + 0.5 mg/l IBA	Hussein *et al*., 2005
Zingiber officinale (Ginger)	Vegetative buds	Shoot, Root	LS + 17.76 µM BAP	Kavyashree, 2009
	Rhizomes bud	Shoot	MS + 4.0 mg/l BAP + 0.05 mg/l NAA	Nkere and Mbanaso, 2010
	Rhizomes bud	Shoot	MS + 2.0 mg/l BAP + 0.5 mg/l NAA	Kambaska and Santilata, 2010
	Rhizomes buds	Root	MS + 2.0 mg/l NAA	Kambaska and Santilata, 2010
Centella asiatica (Pegaga)	Axillary buds	Shoot	MS + 2.0 mg/l BAP	Karthikeyan *et al*., 2009
	Nodal	Shoot	MS + 2.0 mg/l BAP + 0.5 mg/l Kn	Karthikeyan *et al*., 2009
	Shoot tips	Shoot	MS +17.76 µM BAP+1.44 µM GA_3	Sivakumar *et al.*, 2006
	Axillary buds	Root	MS + 1.5 mg/l IBA	Karthikeyan *et al*., 2009
	Shoot tips	Root	½ MS + 10.74 µM NAA	Sivakumar *et al.*, 2006
Justicia gendarussa (Gendarussa)	Nodal	Shoot	MS + 17.7 µM BAP	Thomas and Yoichiro, 2009
	Nodal	Shoot	MS + 3.0 mg/l BAP and 10 % (coconut milk)	Thomas and Yoichiro, 2009
	Leaf	Callus	MS + 13.9 µM Kn + 4.5 µM 2,4-D	Thomas and Yoichiro, 2009
	Shoot	Root	½ MS	Janarthanam and Sumathi, 2010
Kaempferia galanga (Cekur)	Rhizome	Shoot	MS + 2.0 mg/l BAP + 0.2 mg/l NAA	Kalpana and Anbazhagan, 2009
	Rhizome buds	Shoot	MS + 1.0 mg/l BAP + 0.5 mg/l IAA	Parida *et al*., 2010
	Rhizome	Root	½ MS + 1.0 mg/l IBA	Kalpana and Anbazhagan, 2009
Orthoshipon stamineus (Misai kucing)	Leaf	Callus	MS + 1.0 mg/l 2,4-D + 1.0 mg/l NAA	Lee and Chan, 2004a

*MS – Murashige & Skoog, 1962; ½ MS – half strength Murashige and Skoog, (1962); LS- Linsmaier and Skoog, (1965); Kn – Kinetin; BAP- 6-benzylaminopurine; 2,4-D- 2,4-dichlorophenoxyacetic acid; IBA- Indole-3-butyric acid; BA- 6- benzyladenine; NAA- α-naphthalene acetic acid; IAA- 3-indole acetic acid; GA_3- Gibberellic acid.

2.0 TONGKAT ALI

Eurycoma longifolia Jack belongs to Simaroubaceae family, commonly, as Tongkat Ali, the tall plant, slender shrub-tree and found as an under storey in the lowland forest (Hussein *et al.*, 2005). The roots are rich in various bioactive compounds such as eurycomaoside, eurycolactone, eurycomalactone and eurycomanone. Among them, alkaloids and quassinoids have been traditionally used to treat antimalarial, aphrodisiac, antidiabetic, antimicrobial and antipyretic activities (Bhat and Karim, 2010). The most common method for propagation of *E. longifolia* is through seeds however plant regeneration *in vitro* has been successfully established from different plant organs such as leaves, root, nodal, stem, petiole, cotyledon and rachis. Figure 1.1 shows *Eurycoma longifolia* Jack plant.

Figure 1.1 *Eurycoma longifolia* Jack plant. Scale bar: 9.5 cm

2.1 Callus Induction

Callus induction of *E. longifolia* has been reported from leaves, petiole, rachis, stem, tap root, fibrous root, cotyledons and embryo segments when cultured on MS medium (Murashige and Skoog, 1962) supplemented with 2,4-D, picloram, dicamba, NAA and IAA ranging from 1.0 to 6.0 mg L^{-1} (Mahmood *et al.*, 2010). The highest percentage of callus induction (88.33%) was achieved when 2.0 mg L^{-1} 2,4-D was applied.

2.2 Shoot Regeneration

The effect of different types of cytokinins on shoot regeneration of this plant has been intensively studied (such as kinetin, BAP and zeatin). The highest percentage of shoot regeneration was successfully obtained when shoot tips explants were treated with 5.0 mg L^{-1} (90%) and 4.0 mgL^{-1} kinetin (80%). However, 5.0 mg L^{-1} kinetin produced the maximum number of shoots (4.0).

2.3 Root Formation

For root formations, high percentage (90%) of root regeneration was obtained when shoot tips were cultured on MS media supplemented with 0.5 mg L^{-1} IBA after 14 days culture (Hussein *et al.*, 2005). The roots elongated up to 8.0 ± 1.0 cm in length after two months in culture while IBA is superior compared to the auxin. It could be due to facts that IBA is less degraded during autoclaving and stable at room temperature as compared to IAA (Cuenca *et al.*, 1999; Hussein *et al.*, 2005). However, high levels of endogenous auxins or addition of exogenous auxin could cause inhibition of root development in shoots, thus resulting in callus formation at the base of the shoots (Juliani *et al*., 1999; Hussein *et al*., 2005).

3.0 GINGER

Zingiber officinale Roscoe or known as ginger, is a member of Zingiberaceae (Figure 1.2). This plants is an herbaceous perennial and commercially grown for spices, medicine and culinary preparations (Saingproa and Kanchanapoom, 1997; Pandey *et al.*, 1997). [6]-gingerol is the most abundant constituent of ginger and reported to possess substantial antioxidant activity (Purnomo *et al*., 2010). Commonly, ginger is vegetatively propagated via rhizomes. However, its multiplication rate is relatively slow (Nkere and Mbanaso, 2010). Therefore, *in vitro* propagation of ginger offers an efficient technique for obtaining disease-free plant with rapid multiplication and high production of ginger (Kambaska and Santilata, 2009). Micropropagation is also an ideal method for mass propagation of pest and disease free of ginger as compared to conventional method.

A

B

Figure 1.2 *Zingiber officinale* Roscoe plant (A) and Rhizome morphology of *Zingiber officinale* Roscoe (B). Scale bars: 10.5 cm and 5.5 cm, respectively

3.1 Shoot Regeneration

Zingiber officinale can be propagated through *in vitro* by using different types of media and explants. The effects of different types and concentrations of plant growth regulators such as BAP, kinetin and NAA on shoot regeneration have been studied. High percentage of shoot regeneration (95%) is recorded when the explants are cultured on MS media supplemented with 17.76 µM BAP after 15 days culture using vegetative buds (Kavyashree, 2009).

Another assessment demonstrated that 0.05 mg L^{-1} NAA and 4.0 mg L^{-1} BAP produced high mean shoots number (4.0) using leaf explants (Nkere and Mbanaso, 2010). Other type of explants such as fresh rhizome bud produced high shoot regeneration (7.5 ± 0.45 shoots per explants) when the explants were treated with 2.0 mg L^{-1} BAP + 0.5mg L^{-1} NAA (Kambaska and Santilata, 2010).

3.2 Root Induction

Root induction of *Z. officinale* has been established by using various concentrations and types of plant growth regulators. Kavyashree (2009) reported high mean number of roots was obtained (12.3) when vegetative buds were cultured on Linsmaier & Skoog (LS) medium supplemented with 17.76 µM BAP. However, the highest percentage of root induction (95%) with an average number of 8.5 roots ± 0.33 per explants were recorded on half strength MS medium supplemented with 2.0 mg L^{-1} NAA by using fresh rhizome bud explants (Kambaska and Santilata, 2010).

■4.0 PEGAGA

The genus *Centella* (Umbellifarae) comprises 33 plant species. *Centella asiatica* L. or commonly known as pegaga is found in tropical and sub-tropical countries (Figure 1.3). Tiwari *et al*., 2000 reported that, *C. asiatica* extracts were used for treatment of asthma, bronchitis, dropsy, elephantiasis, gastric catarrh, kidney troubles, leprosy, leucorrhoea, skin disease and urethritis. Besides that, *C. asiatica* plants are also reported to contain glycosides groups such as indocentelloside, brahmoside, brahminoside, asiaticoside, theankuniside and isothankuniside.

Asiaticoside is the major compound presents in *C. asiatica* and used to treat leprosy and tuberculosis (Tiwari *et al.*, 2000) while Karthikeyan *et al*., (2009) reported that whole plants exhibited antibacterial, antiinflammatory, antifebrile and antigalactogogic activities. Micropropagation of *C. asiatica* for production of pesticide–free plants (Sivakumar *et al.*, 2006), rapid clonal propagation of elite clones and germplasm conservation of *C. asiatica* has been established (Tiwari *et al*., 2000).

Figure 1.3 *Centella asiatica* L plant. Scale bar: 9.3 cm

4.1 Shoot Regeneration

In vitro plant regeneration of *C. asiatica* has been established from various sources such as stem node (Hossain *et al*., 2000) and somatic embryos (Martin, 2004). Shoot formation was successfully induced from axillary buds growth on MS medium supplemented with 2.0 mg L^{-1} BAP after 4 weeks in culture (Karthikeyan *et al.*, 2009). However, the combination of 2.0 mg L^{-1} BAP and 0.5 mg L^{-1} kinetin produced maximum of 18 shoots per explants. Multiple shoots (16.8 shoots/explants) were also successfully induced when shoot tip explants were cultured on MS-based media supplemented with a combination of 17.76 µM BAP + 1.44 µM GA_3 (Sivakumar *et al*., 2006).

4.2 Root Induction

Various auxins such as IBA, IAA and NAA have influenced on percentage of root induction in *C. asiatica*. The frequency of roots produced depends on type and concentrations of the auxins used. For examples, 1.5 mg L^{-1} IBA produced maximum number of roots (12 roots per explant) after 30 days culture (Karthikeyan *et al.*, 2009). However, about 90% of shoot explants produced 18 – 19 roots per explants when placed on half-strength MS plates supplemented with 10.74 µM NAA (Sivakumar *et al.*, 2006).

4.3 Somatic Embryogenesis and Suspension Cell Culture

There are few reports on somatic embryogenesis studies of *C. asiatica*. Suspension cell culture of *C. asiatica* has been established from leaf and internode-derived calluses cultured in half–strength MS liquid medium containing 2.69 mM NAA and 1.16mM kinetin (Martin, 2004). Other factors, such as sucrose concentration, IAA and BAP influence establishment of cell suspension culture (Omar *et al.*, 2004). Increment of sucrose from 3.32% to 6.68% (w/v) causes dry cell weight in *C. asiatica* increases from 16 to 27 g L^{-1}, respectively. The optimum dry cell weight is achieved at treatment of 6.68% (w/v) sucrose, 0.84 mg L^{-1} IAA and 1.17 mg L^{-1} BAP (27.4 g L^{-1} dry cell weight).

■5.0 GANDARUSA

Justicia gendarussa Burm. f or commonly known as Gendarusa is a member of Acanthaceae family and found abundantly in many countries including Indonesia, India and Malaysia (Figure 1.4). Traditionally, Gandarusa extracts have been used to treat ailments such as emetic, antipyretic, amenorrhea, stomach troubles, hemoptysis, cough and asthma (Khatijah and Noraini, 2007). Some flavonoids act as bioactive compounds such as antioxidant, antiinflammatory and antianalgestic activities (Jothimanivannan *et al.*, 2010).

Figure 1.4 *Justicia gendarussa* Burm. f plant. Scale bar: 9.4 cm

5.1 Callus Induction

Induction of callus leaf explants has been demonstrated by using different concentrations of 2,4-D and Kinetin. However, a combination of 13.9 µM kinetin and 4.5 µM 2,4-D showed high callus induction, up to 78% (Thomas and Yoichiro, 2010).

5.2 Shoot Regeneration

Gandarusa is easily propagated by stem cutting but produced low mass propagation (Musa *et al.*, 2009). Alternatively, tissue culture system allows rapid, consistently supply of plant materials and mass production of genotypically stabled of *J. gendarussa*. Plant regeneration of *J. gendarrussa* has been established from callus (Thomas and Yuichiro, 2010). Thomas and Yoichiro (2010) reported that high percentage of shoots (87%) were induced from nodal explants when cultured on media supplemented with 17.7 µM BAP. Induction of 10% coconut milk in media also induced multiple shoot (4.3) was reported (Janarthanam and Sumathi, 2010).

5.3 Root Formation

The addition of auxin (IBA and NAA) and half-strength MS have influenced root induction in *J. gendarussa*. IBA (9.8 µM) induces high percentage of root regeneration from shoot explants (73%) (Thomas and Yoichiro, 2010). However, roots could also be obtained by culturing shoots in half strength MS medium without growth regulators (Janarthanam and Sumathi, 2010).

■6.0 CEKUR

Kaempferia galanga L. or known as 'Cekur' belongs to family Zingiberaceae (Figure 1.5). This plant is an aromatic perennial herb and has aromatic rhizomes and leaves (Mohanty *et al*., 2011). It is distributed in Southern China, Indochina, Malaysia, India and Thailand (Chirangini *et al*., 2005; Hanumantharaju *et al*., 2010). The leaves of *K. galanga* are used in flavouring foodstuffs, hair tonics, mouth washes and cosmetic industries (Parida *et al*., 2010). Whereas, the rhizome parts containing the essential oils are used as decoction or powder for indigestion, cold, pectoral and abdominal pains, headache and toothache (Kanjanapothi *et al*., 2004). Volatile oils of dried rhizome of *K. galanga* have been demonstrated anti-microbial activity against some Gram positive and Gram negative bacteria (Tewtrakul *et al.*, 2005). *K. galanga* is normally propagated by rhizomes (Rahman *et al*., 2005). However, the conventional propagation of *K. galanga* by splitting of rhizomes is slow and resulted in insufficient to meet market demand (Kalpana and Anbazhagan, 2009**).** Therefore, tissue culture approach can be used as an alternative to propagate the plants rapidly and in large quantities.

Figure 1.5 *Kaempferia galanga* L plant. Scale bar: 9.5 cm

6.1 Shoot Regeneration

Shoot regeneration in this plant has been reported such as in Kalpana and Anbazhagan, 2009. High number of shoots (19.4 shoots per explant) and percentage of shoot regeneration (85%) were achieved when rhizome explants was treated with 2.0 mg L^{-1} BAP and 0.2 mg L^{-1} NAA. A combination of 1 mg L^{-1} BAP and 0.5 mg L^{-1} IAA also induces high rate of shoot multiplication (11.5 ± 0.6 shoots/ lateral rhizome bud explants) as well as leaf biomass production (7.4 g/explants) (Parida *et al.*, 2010).

6.2 Root Induction

Rooting could be induced by culturing shoot explants in half strength of MS medium containing auxins such as NAA and IBA. Among auxins, IBA (1.0 mgL^{-1}) promotes high percentage of root regeneration (96%) (Kalpana and Anbazhagan, 2009).

■7.0 MISAI KUCING

Orthosiphon stamineus, known as Misai kucing (Cat's Whiskers, Java Tea) is a member of Lamiacease family (Figure 1.6). It is a popular medicinal herbs in South East Asia including Malaysia and Singapore. These plants grow well on wet soil and can be found in both temperature and tropical gardens (Hsuan, 1986). In Europe and Japan, the leaves are used as herbal tea. It is believed that a diuretic activity in plant extracts can cause removal of uric acid stones from kidney. It is also used for treatment of diabetes and hypertension (Mat-Salleh and Latif, 2005). These properties such as anti-diuretic and antibacterial activities could be due to high flavonoids and phenolic compounds present in *O. stamineus* (Schut and Zwaving, 1993).

Figure 1.6 *Orthosiphon stamineus* Benth plant. Scale bar: 8.5 cm

7.1 Shoot Regeneration

Plant regeneration of *O. stamineus* from nodal segments has been reported by Lee and Chan (2004a). Multiple shoots (6.1 shoots per explants) was observed from stem nodal explants when treated in MS media with supplemented 6.7 µM BAP in 4 weeks culture.

7.2 Suspension Cell Culture

Establishment of suspension cell culture of *O. stamineus* from callus has been reported (Lee and Chan, 2004b). For callus induction, the highest percentage of callus production is obtained when leaf explants are cultured on MS medium supplemented with 1.0 mg L^{-1} 2,4-D and 1.0 mg L^{-1} NAA. The friable callus is used to initiate suspension cell culture. Lee and Chan (2004b) reported that 0.75g inoculums cell in 20 mL MS liquid medium supplemented with 1.0 mg L^{-1} 2,4-D was the best condition for cell suspension culture of *O. stamineus*. The optimum cell culture growth was maintained by subculturing fortnightly.

■8.0 CONCLUSION

In conclusion, this paper could serve as an encouragement and knowledge for other researchers to further works on *in vitro* propagation of local medicinal plants in Malaysia. *In vitro* cultures allow conservation of rare and endangered plant from in discrimination and unsustainable harvesting in the wild that considered important in preserving the malay folklore medicine.

Acknowledgement

The authors would like to record their gratitude to Hj Mohd Ali Hj Mokti for the pictures of some medicinal plants.

References

[1] Bhat, R. and Kasim, A. A. 2010. Tongkat Ali (*Eurycoma longifolia* Jack): A Review on Its Ethnobotany and Pharmacological Importance. *Fitoterapia*. 81: 669–679.

[2] Bourgaud, F., Gravot, A., Milesi, S. and Gontier, E. 2001. Production of Plant Secondarymetabolites: A Historical Perspective. *Plant Science*. 161: 839–851.

[3] Chirangini, P., Sinha, S.K. and Sharma, G.J. 2005. *In Vitro* Propagation and Microrhizomes Induction in *Kaempferia Galanga* Linn. and *K. rotundo* Linn. *Indian Journal of Biotechnology*. 4: 404–408.

[4] Cuenca, S., Amo-Marco, J.B. and Parra, R .1999. Micropropagation from Inflorescence Stems of the Spanish Endemic Plant *Centaurea paui* Loscos ex Willk. (Compositae). *Plant Cell Reports.* 18: 674–679.

[5] Forkman, G. 1991. Flavonoids Flower Pigments: The Formation of the Natural Spectrum Andits Extension by Genetic Engineering. *Plant Breeding*. 106: 1–29.

[6] Hanumantharaju, N., Shashidhara, S., Rajasekharan, P.E. and Rajendra, C.E. 2010. Comparatived Evaluation of Antimicrobial and Antioxidant Activities of Kaempferia Galanga for Natural and Micropropagated Plant. *International Journal of Pharmacy and Pharmaceutical Sciences*. 2(4): 72–75.

[7] Hussien, S., Ibrahim, R., Kiong, A. L. P., Mohd Fadzillah, N. and Daud, S. K. 2005. Multiple Shoot Formation of an Important Tropical Medicinal Plant, *Eurycoma Longifolia* Jack. *Plant Biotechnology*. 22: 349–351.

[8] Hossain, S. N., Rahman, S., Joydhar, A., Islam, S. and Hossain, M. 2000. *In Vitro* Propagation of Thankuni (*Centella asiatica* L.). *Plant Tissue Culture*. 10: 17–23.

[9] Hsuan, K. 1986. *Order and Famili Tumbuhan Berbiji di Tanah Melayu*. Dewan Bahasa dan Pustaka, Kuala Lumpur, Malaysia. 393–397.

[10] Janarthanam, B. and Sumathi, E. 2010. *In Vitro* Regeneration of *Justicia gendarussa* Burm. f. *Libyan Agriculture Research Center Journal Internation*. 1(5): 284–287.

[11] Jothimanivannan, C., Kumar, R. S. and Subramanian, N. 2010. Antiinflammatory and Analgesic Activities of Ethanol Extract of Aerial Parts of *Justicia Gendarussa* Burm. *International Journal of Pharmacology*. 6: 278–283.

[12] Juliani, H. R., Koroch, A. R., Juliani, H. R. and Trippi, V. S. 1999. Micropropagation of *Lippia junelliana* (Mold.) Trone. *Plant Cell, Tissue and Organ Culture*. 59: 175–179.

[13] Kalpana, M. and Anbazhagan, M. 2009. *In vitro* Production of *Kaempferia Galanga* (L).- An Endangered Medicinal Plant. *Journal of Phytology*. 1(1): 56–61.

[14] Kambaska, K. B. and Sanikata, S. 2009. Effect of Plant Growth Regulator on Micropropagation of Ginger (Zingiber offirale Rosc.) cv- Suprava and Suruchi. *Journal of Agricultural Technology*, **5**(2):271–280.

[15] Kanjanapothi, D., Panthong, A., Lertprasertsuke, N., Taesotikul, T., Rujjanawate, C., Kaewpinit, D., Sudthayakor, R., Choochote, W., Chaithong, U., Jitpakdi, A. And Pitasawat, B. 2004. Toxicity of Crude Rhizome Extract of *Kaempferia galanga* L. (Proh Hom). *Journal of Ethnopharmacology*. 90: 359–365.

[16] Karthikeyan, K., Chandran, C. and Kulothugan, S. 2009. Rapid Clonal Multiplication Through *In Vitro* Axillary Shoot Proliferation of *Centella asiatica* L. *Indian Journal of Biotechnology*. 8: 232–235.

[17] Kavyashree , R. 2009. An Efficient *In Vitro* Protocol for Clonal Multiplication of Ginger–Vir Varada. *Indian Journal Biotechnology*. 8: 328–331.

[18] Khatijah, H. and Noraini, T. 2007. *Anatomical Atlas of Medicinal Plants*. Penerbit UKM. 1: 106.

[19] Kuo, P. C., Shi, L. S., Damu, A. G., Su, C. R., Huang, C. H., Ke, C. H., Wu, J. B., Linn, A. J., Bastow, K. F., Lee, K. H. and Wu, T. S. 2003. Cytotoxic and Antimalarial-carboline Alkaloids from the Roots of *Eurycoma longifolia*, *J.Nat.Prod*. 66: 1324–1327.

[20] Lee, W. L. and Chan, L. K. 2004a. Plant Regeneration from Stem Nodal Segments of *Orthosiphon Stamineus* Benth, A Medicinal Plant with Diuretic Activity. *In vitro Cell and Development Biology- Plant*. 40: 115–118.

[21] Lee, W. L. and Chan, L. K. 2004b. Establishment of *Orthosiphon Stamineus* Cell Suspension Culture for Cell Growth. *Plant Cell Tissue and Organ Culture*. 78: 101–106.

[22] Linsmaier, E. M. and Skoog, F. O. 1965. Organic Growth Factor Requirements of Tobaccotissue Culture. *Physiologia Plantarum*. 18: 100–127.

[23] Mahmood, M., Normi, R. And Subramaniam, S. 2010. Optimization of Suitable Auxin Application in a Recalcitrant Woody Forest Plant of *Eurycoma Longifolia* (Tongkat Ali) for Callus Inducation. *African Journal of Biotechnology*. 9(49): 8417–8428.

[24] Manoj, G., Sasmal, D. And Nagori, B. P. 2011. Review on Medicinal Plants Used by Local Community of Jodhpur District of Thar Desert. *International Journal of Pharmacology*. **7**(3): 333–339.

[25] Mat-Salleh, K. and Latiff, A. 2005. *Tumbuhan Ubatan Malaysia*. Universiti Kebangsaan Malaysia, Bangi, Malaysia. 254.
[26] Martin, K. P. 2004. Plant Regeneration Through Somatic Embryogenesis in Medicinally Important *Centella asiatica* L. *In vitro Cell Development Biology- Plant*. 40: 586–591.
[27] Mohanty, S., Parida, R., Singh, S., Joshi, R. J. and Subudhi, E., Nayak, S. 2011. Biochemical and Molecular Profiling of Micropropagated and Conventionally Grown *Kaempferiagalanga* L. *Plant Cell, Tissue and Organ Culture*. 106(1): 39–46.
[28] Murashige, T. and Skoog. F. 1962. A Revised Medium for Rapid Growth and Bioassays with Tobacco Tissue Cultures. *Physiologia Plantarum*. 15: 473–479.
[29[Musa, Y., Azimah, A.K. and Zaharah, H. 2009. *Tumbuhan Ubatan Popular Malaysia*. Institut Penyelidikan dan Kemajuan Pertanian. 1: 40.
[30] Nkere, C. K. and Mbanaso, E. N. A. 2010. Optimizing Concentrations of Growth Regulators for *In Vitro* Ginger Propagation. *Journal of Agrobiology*. 27(2): 61–65.
[31] Omar, R., Abdullah, M.P., Hasan, M, A. and Marziah, M. 2004. Development of Growth Medium for *Centella Asiatica* Cell Culture Via Responce Surface Morphology. *American Journal of Applied Science.* 1(3): 215–219.
[32] Pandey, Y. R., Sagnansupyakorn, C., Sabarachanin, O. Nd Thareechai, N. 1997. *In Vitro* Propagation of Ginger (*Zingeber Officinale Roscoe*). *Kasetsart Journal.* 31(1): 81–86.
[33] Parida, R., Mohanty, S., Kuanar, A. and Nayak, S. 2010. Rapid Multiplication and *In Vitro* Production of Leaf Biomass in *Kaempferia Galanga* Through Tissue Culture. *Electronic Journal of Biotechnology*. 13(4): 1–8.
[34] Purnomo, H., Jaya, F. and Widjanarko, S. B. 2010. The Effects of Type and Time of Thermal Processing on Ginger (*Zingiber Officinale* Roscoe) Rhizome Antioxidant Compounds and Its Quality. *International Food Research Journal.* 17: 335–347.
[35] Rahman, M. M., Amin, M. N., Ahamed, T., Habib, A., Ahmed, R., Ahmed, M. B. and Ali, M. R. 2005. *In Vitro* Rapid Propagation of Black Thorn (*Kaempferia Galanga* (L): A Rare Medicinal and Aromatic Plant of Bangladesh. *Journal of Biological Sciences*. 5(3):300–304.
[36] Rafidah, S., Suryani, A., Johari, R. and Radzali, M. 2004. Callus Induction on a Basal Medium of Murashige and Skoog Supplemented with Plant Growth Regulator by Using Different Explants of *Citrus Grandis* L. Osbeck. *Malaysian Society of Plant Physiology*. 13: 207–213.
[37] Reza, B., Omid, K. and Mansour, G. 2009. Influence of Carbon Sources and Their Concentrations on Rooting and Hyperhydricity of Apple Rootstock MM.106. *World Applied Sciences Journal*. 6(11): 1513–1517.
[38] Rout, G. R., Samantaray, S. and Das, P. 2000. *In Vitro* Manipulation and Propagation of Medicinal Plants. *Biotechnology Advances*. 18: 91–120.
[39] Saingproa, B. and Kanchanapoom, K. 1997. Clonal Propagation Through Multiple Shoot Formation from Ginger (Zingiber Offinale Roscoe) Callus and Buds. *Suranaree Journal of Science and Technology*. 4: 1–5.
[40] Schut, G. A. and Zwaving, J. H. 1993. Pharmacological Investigation of Some Lipophilic Flavonoids from *Orthosiphon aristatus*. *Fitoterapia*. 64: 99–102.
[41] Sivakumar, G., Alagumanian, S. and Rao, M.V. 2006. High Frequency *In Vitro* Multiplication of *Centella Asiatica* : An Important Industrial Medicinal Herbs. *Engineering in Life Sciences*. 6(6): 597–601.
[42] Suneetha, M. S. and Chandrakanth, M. G. 2006. Establishing a Multi-Stakeholder Value Indexin Medicinal Plants—An Economic Study on Selected Plants in Kerala and Tamil Nadu States of India. *Ecological Economics*. 60(1): 36–48.
[43] Tewtrakul, S., Yuenyongsawad, S., Kummee, S., and Atsawajaruwan, L. 2005. Chemical Components and Biological Activities of Volatile Oil of *Kaempferia Galanga* Linn. *Songklanakarin Journal of Science and Technology*. 27(2): 503–507.
[44] Thomas, T. D. and Yoichiro, H. 2009. *In Vitro* Propagation For The Conservation Of A Rare Medicinal Plant *Justicia Gendarussa* Burm. F. By Nodal Explants And Shoot Regeneration From Callus. *Acta Physiologiae Plantarum*, **32** (5):943-950.
[45] Tiwari, K. H., Shama, N. C., Tiwari, V. and Singh, B. D. 2000. Micropropagation of *Centella asiatica* (L.), a Valuable Medicinal Herb. *Plant Cell, Tissue and Organ Culture*. 63:179–185.

.